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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/829,250	04/22/2004	Noriaki Hattori	252040US0C0CONT	8196
2292 7590 01/11/2008 BIRCH STEWART KOLASCH & BIRCH PO BOX 747 FALLS CHURCH, VA 22040-0747			EXAMINER SLOBODYANSKY, ELIZABETH	
			ART UNIT 1652	PAPER NUMBER
			NOTIFICATION DATE 01/11/2008	DELIVERY MODE ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

mailroom@bskb.com

Office Action Summary	Application No. 10/829,250	Applicant(s) HATTORI ET AL.	
	Examiner Elizabeth Slobodyansky, PhD	Art Unit 1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☒ Responsive to communication(s) filed on 13 December 2007.
- 2a) ☐ This action is **FINAL**. 2b) ☒ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 4) ☒ Claim(s) 35,37,40 and 42 is/are pending in the application.
- 4a) Of the above claim(s) _____ is/are withdrawn from consideration.
- 5) ☐ Claim(s) _____ is/are allowed.
- 6) ☒ Claim(s) 35,37,40 and 42 is/are rejected.
- 7) ☐ Claim(s) _____ is/are objected to.
- 8) ☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on _____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All b) ☐ Some * c) ☐ None of:
1. ☐ Certified copies of the priority documents have been received.
 2. ☐ Certified copies of the priority documents have been received in Application No. _____.
 3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).
- * See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

- | | |
|--|---|
| 1) <input type="checkbox"/> Notice of References Cited (PTO-892) | 4) <input type="checkbox"/> Interview Summary (PTO-413)
Paper No(s)/Mail Date. _____ |
| 2) <input type="checkbox"/> Notice of Draftsperson's Patent Drawing Review (PTO-948) | 5) <input type="checkbox"/> Notice of Informal Patent Application |
| 3) <input type="checkbox"/> Information Disclosure Statement(s) (PTO/SB/08)
Paper No(s)/Mail Date _____ | 6) <input type="checkbox"/> Other: _____ |

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on December 13, 2007 has been entered.

The amendment filed December 13, 2007 amending the specification to correct typographical errors, canceling claims 14-18, 20-24 and 32 and amending claims 33-35, 37 and 40 and adding claim 42 has been entered.

The Declaration under 37 CFR 1.132 by Dr. Seiji Murakami filed December 13, 2007 has been entered.

Claims 33-35, 37, 40 and 42 are pending. Claims 33 and 34 have been previously withdrawn.

Specification

The disclosure is objected to because of the following: It was amended on December 13, 2007 to recite "the mutant luciferase is a polypeptide comprising an amino acid sequence shown in SEQ ID NO: 4 or 6 or 8 or said amino acid sequence wherein one or more amino acids are added, deleted or substituted". SEQ ID NO:8 is

the amino acid sequence of naturally occurring HEIKE (*Luciola lateralis*) luciferase not a mutant luciferase.

Appropriate correction is required.

Claim Objections

Claims 37, 40 and 42 are objected to because they do not comply with the Sequence Rules 37 CFR 1.821 through 1.825.

37 CFR 1.821(d) requires the use of assigned sequence identifier in all instances where the description or claims of a patent application discuss sequences containing 4 or more specifically defined amino acids. Said sequences should be present in the Sequence listing and computer readable form thereof.

It is noted that Applicants intend to file a substitute Sequence Listing at a later time (Remarks 12/13/07, page 6). The objection is maintained until the substitute Sequence Listing is filed.

Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 37, 40 and 42 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as

to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

With regard to claim 37, Applicants indicate support for the amino acid sequence PXAVVVLX₄₉₀GKXMTE, in which X₄₉₀ is an amino acid other than glutamic acid and X is any amino acid stating that "the short sequence recited remains variable at two positions other than position 490, however, the mutant enzyme is recited as one that is a firefly luciferase. The two additional positions that are variable in claim 37 are those that demonstrate variation among the species disclosed in the instant sequence listing" (Remarks of 12/13/07, page 8). This is not agreed with because while there is support for an amino acid other than glutamic acid at position 490 (specification, page 3, lines 8-11), there is no support for two additional positions that are G and S, respectively, that are G and S in both SEQ ID NOs: 4 and 6, as well as no support for the amino acid sequence that corresponds to the specific fragment of positions 483-497 in both SEQ ID NOs: 4 and 6. With regard to claims 40 and 42 that recite the amino acid sequence PGAVVVLX₄₉₀GKSMTE, in which X₄₉₀ is an amino acid other than glutamic acid, Applicants argue that "The Examiner cannot reasonably assert that a skilled molecular biologist is unable to visualize the possible variant amino acids that might be placed at that position. The disclosure of the present application makes quite clear that the position 490 is a determinant of surfactant resistance and that enzymes having an amino acid other than glutamic acid at this position are resistant to surfactant. See, e.g. page 3, lines 8-11 of the specification" (Remarks of 12/13/07, page 7). This is not agreed with because while molecular biologist would be able to visualize the possible

variant amino acids that might be placed at position 490, the rejection is over the support for the specific fragment recited in the claims.

Thus there is no indication that mutant luciferases of claims 37, 40 and 42 were within the scope of the invention as conceived by Applicants at the time the application was filed.

Accordingly, Applicants are required to cancel the new matter in the response to this Office Action.

Claims 37 and 42 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claim 37 is drawn to a firefly luciferase retaining at least 85% activity in the presence of 0.1% surfactant and comprising the sequence PXAVVVLX₄₉₀GKXMTE, in which X₄₉₀ is an amino acid other than glutamic acid and X is any amino acid. Claim 42 depend from claim 37 and limits the sequence to PGAVVVLX₄₉₀GKSMTE, in which X₄₉₀ is an amino acid other than glutamic acid.

It is unclear how the term "firefly luciferase" limits the structure of the luciferase, when it describes a mutant luciferase in fact. Thus, the claims are construed as drawn to a genus of mutant luciferase retaining at least 85% activity in the presence of 0.1% surfactant comprising a short sequence. Because, said sequence does not exhibit

luciferase properties and the rest of the structure is not described, said genus of mutant firefly luciferase is characterized by function.

Applicants disclose two mutants of *L. lateralis* luciferase having an improved activity in the presence of a surfactant having sequences of SEQ ID NOs: 4 and 6 that comprise the fragment PGAVVVLKGKSMTE (positions 483-497 in both SEQ ID NOs: 4 and 6), said fragment comprising mutation E490K. (These two sequences differ by the mutation at position 217 wherein SEQ ID NO:4 has A217L and SEQ ID NO:6 has A217I). Therefore, a representative number of firefly luciferases retaining at least 85% activity in the presence of 0.1% surfactant and comprising the requisite sequence with mutation at position 490 is two. Moreover, the specification fails to describe any other representative species by any identifying characteristics or properties other than the "functionality" of having an improved activity in the presence of a surfactant and fails to provide any structure: function correlation present in all members of the claimed genus. Therefore, the specification is insufficient to put one of skill in the art in possession of the attributes and features of all species within the claimed genus. Therefore, one skilled in the art cannot reasonably conclude that the applicant had possession of the claimed invention at the time the instant application was filed.

Claims 35 and 40 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a fragment of luciferase comprising E490K, does not reasonably provide enablement for a fragment of luciferase comprising E490X, in which X is not K as well as the full length luciferase comprising either E490K or E490X.

The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or the invention commensurate in scope with these claims.

Claim 35 is drawn to a luciferase retaining more than 85% of its luciferase activity in 0.1% benzalkonium chloride compared to its luciferase activity in the absence of benzalkonium chloride obtained by amplifying a template nucleic acid prepared from GENJI firefly or HEIKE firefly using primers of SEQ ID NOs: 1 and 2. Claim 40 depends from claim 35 and limits the luciferase to comprising the sequence PGAVVVLX₄₉₀GKSMTE, in which X₄₉₀ is an amino acid other than glutamic acid.

It appears that SEQ ID NO:1 is a mutagenic primer resulting in obtaining a fragment with mutation E490K. It is unclear to which sequence corresponds primer of SEQ ID NO:2.

The use of primers of SEQ ID NOs: 1 and 2 could possibly allow for obtaining only a luciferase fragment comprising position mutation E490K. therefore, the use of SEQ ID NOs: 1 and 2 does not allow to obtain a fragment comprising any mutation other than E490K, i.e. does not allow to obtain a fragment comprising E490X as required by claim 40 and obtaining the full length active luciferase comprising either E490K or E490X. Furthermore, in order to obtain said mutants residues 483-497 in the wild-type luciferase should be replaced with the amplified fragment.

Thus, applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make a full length mutant GENJI or HEIKE luciferases using primers of SEQ ID NOs: 1 and 2 in a manner reasonably correlated with the scope of the claims.

The scope of the claims must bear a reasonable correlation with the scope of enablement (In re Fisher, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, making said full length luciferases having the desired biological characteristics is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See In re Wands 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988).

Claims 37 and 42 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for mutant *Luciola lateralis* luciferases having the sequences of SEQ ID NO:4 or SEQ ID NO:6 that that have the mutation at position 490 and have at least 85% activity in the presence of 0.1% surfactant compared to the native luciferase and for mutant *Luciola cruciata* luciferases with corresponding sequences, does not reasonably provide enablement for a firefly luciferase retaining at least 85% activity in the presence of 0.1% surfactant and comprising the requisite fragment with mutation at position 490 and having an unknown homology to SEQ ID NO:4 or SEQ ID NO:6, the specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, how to make the invention commensurate in scope with these claims.

Claim 37 is drawn to a firefly luciferase retaining at least 85% activity in the presence of 0.1% surfactant and comprising the sequence PXAVVVLX₄₉₀GKXMTE, in which X₄₉₀ is an amino acid other than glutamic acid and X is any amino acid. Claim 42

depend from claim 37 and limits the sequence to PGAVVVLX₄₉₀GKSMTE, in which X₄₉₀ is an amino acid other than glutamic acid.

Claims 37 and 42 are so broad as to encompass any mutant luciferase with an unknown homology to the luciferase of *Luciola lateralis* having the requisite properties in which the amino acid comprises the short fragment wherein residue 490 is substituted compared to a wild type *Luciola* luciferase. The scope of the claims is not commensurate with the enablement provided by the disclosure with regard to the extremely large number of mutant luciferase enzymes broadly encompassed by the claims. Since the amino acid sequence of a protein determines its structural and functional properties, predictability of which changes can be tolerated in a protein's amino acid sequence and obtain the desired activity requires a knowledge of and guidance with regard to which amino acids in the protein's sequence, if any, are tolerant of modification and which are conserved (i.e., expectedly intolerant to modification), and detailed knowledge of the ways in which the proteins' structure relates to its function. However, in this case the disclosure is limited to the nucleotide and amino acid sequence of two mutant luciferases having one or two amino acids different compared with the wild-type sequence.

While recombinant and mutagenesis techniques are known, it is not routine in the art to screen for multiple substitutions or multiple modifications, as encompassed by the instant claims, and the positions within a protein's sequence where amino acid modifications can be made with a reasonable expectation of success in obtaining the desired activity/utility are limited in any protein and the result of such modifications is

unpredictable. In addition, one skilled in the art would expect any tolerance to modification for a given protein to diminish with each further and additional modification, i.e. multiple substitutions. The specification does not support the broad scope of the claims which encompass any mutant luciferase having the requisite property with an undisclosed homology to a *Luciola* luciferase in which the amino acid corresponding to residue 490 of a *Luciola* luciferase is mutated because the specification does not establish: (A) regions of the protein structure which may be modified without effecting luciferase activity; (B) the general tolerance of luciferases to modification and extent of such tolerance; (C) a rational and predictable scheme for modifying any luciferase residues with an expectation of obtaining the desired biological function; and (D) the specification provides insufficient guidance as to which of the essentially infinite possible choices is likely to be successful.

While it is possible to align the sequences, it is unpredictable what effect will have the mutation at position corresponding to position 490 in HEIKE luciferase, if the aligned sequence is not highly identical to the sequence of HEIKE luciferase.

As mentioned above, the sequence PXAVVVLX₄₉₀GKXMTE does not exhibit luciferase properties. The effect of the rest of the luciferase structure is unpredictable and there is no guidance provided as to what the rest of the structure should be.

Thus, applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims broadly including any number of amino acid modifications of any firefly luciferase with no or low homology to a *Luciola* luciferase having the

Art Unit: 1652

desired properties in which the amino acid corresponding to residue 490 is or is not mutated. The scope of the claims must bear a reasonable correlation with the scope of enablement (In re Fisher, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, making mutant firefly luciferases having the desired biological characteristics is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See In re Wands 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988).

The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 35, 37, 40 and 42 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 35, with dependent claim 40, is confusing because primer of SEQ ID NO;1 appears to be a mutagenic not amplifying primer. Furthermore, it appears that claim 35 is incomplete as omitting essential steps, such as the step of ligating the obtained fragment into the plasmid encoding the full-length sequence of the luciferase, for example.

Claim 37, with dependent claim 42, is confusing as drawn to "A firefly luciferase" wherein a mutant luciferase obtained from a firefly luciferase appears to be intended.

Response to Amendment

The Declaration by Dr. Seiji Murakami under 37 CFR 1.132 filed December 13, 2007 is sufficient to overcome the rejection of claims 14-18, 20-24, 32 and 35-41 based upon Hirokawa et al (US Patent 6,074,859, form PTO-1449 filed April 22, 2004).

Dr. Murakami stated that luciferase of SEQ ID NO:14 comprising mutation E490K in *Luciola* luciferase is disclosed but not claimed in the patent (Declaration, page 2, clause 8). He further declared he is the person who designed the experiment and determined the sequence of SEQ ID NO:14, and therefore, conceived and reduced to practice the mutant enzyme (page 3, clause 9).

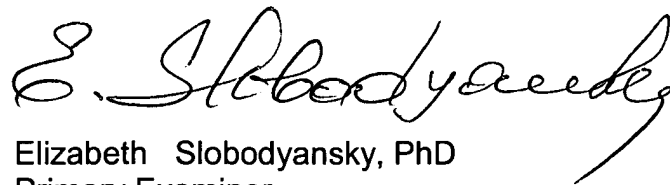
Response to Arguments

Applicant's arguments filed December 13, 2007 have been fully considered and are responded above in the rejections or in the previous Office action mailed June 14, 2007 when they are in the essence the repetition of the previously presented arguments.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Elizabeth Slobodyansky, PhD whose telephone number is 571-272-0941. The examiner can normally be reached on M-F 10:00 - 6:30. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapura Achutamurthy, PhD can be reached on 571-272-0928. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Art Unit: 1652

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A handwritten signature in black ink, appearing to read "E. Slobodyansky", with a long, sweeping flourish extending from the end of the name.

Elizabeth Slobodyansky, PhD
Primary Examiner
Art Unit 1652

January 4, 2007